IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Dario NERI et al.

Examiner: Virginia Allen PORTNER

Serial No.: 10/321,558

Group Art Unit: 1645

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Confirmation No: 9183

Title: SPECIFIC BINDING MOLECULES FOR SCINITIGRAPHY

DECLARATION UNDER 37 C.F.R. §1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Dario Neri, declare that:

1. I am the same Dario Neri who signed the STATEMENT UNDER 37 C.F.R. § 1.804(b) dated October 3, 2008. I am filing this declaration to supplement this prior document.

2. As confirmed in the prior Statement, the DNA contained in deposit no. PTA-9529 (ATCC (American Type Culture Collection, Manassas, VA)) is the same as the DNA of biological material which was in existence prior to the filing date of the first filed ancestor application (09/075,338 of May 11, 1998 (hereinafter "Filing Date")) (i.e., the DNA in the deposit has the same sequence as the DNA in existence before the Filing Date).

3. The deposited DNA encodes an antibody termed "L19" in my prior statement. The biological material in existence before the Filing Date was the same antibody and also had and still has this same nomenclature, "L19." This is because the DNA sequences are identical, of course. This same nomenclature is used in the above-identified application (and throughout its

full parentage) to name the antibody prepared in Example 2 of the application. Example 2 contains a description of the actual procedural steps used before the Filing Date to prepare the "L19" antibody in existence before the Filing Date. For this reason, "L19" is the name given to the product of the procedures reported in Example 2.

4. However, the sequence of the actual L19 (i.e., the actual antibody prepared using the actual Example 2 procedures carried out in our laboratory prior to the Filing Date) described in the above-identified application contained errors. There was not and has never been any actual antibody prepared which contains the erroneous sequence disclosed in the above-identified application. The actual L19 antibody in existence prior to the Filing Date, as stated above, had the same sequence as that contained in the DNA of the PTA-9529 deposit. The actual sequence and the deposited sequence, being the same, are both different from the sequence erroneously described in the above-identified application. This actual sequence of the L19 antibody prepared before the Filing Date (and thus that of the material of deposit PTA-9529) is shown in the attached sequence description, "L19-Sequence."

5. The very same sequence errors contained in the application were also contained in reports of our L19 work in publications and public databases, including EMBL.

6. The two errors from the actual sequence of L19 contained in both the above-identified application and in such other public disclosures are: (1) describing the linker as having a length of 14 amino acids, because an extra two non-existent amino acids (TG) were reported at one end of the linker; and (2) describing the VL sequence as containing tyrosine (Y) in position 32 instead of the correct amino acid which is phenylalanine (F).

7. To ensure clarity: the incorrect 14 amino acid long linker described in the application is not and has never been part of the actual antibody, L19, prepared prior to the Filing Date. It has also never been part of the actual predecessor antibodies mentioned in the specification, E1 and H10. The actual linker length for L19 (and E1 and H10) is and has always been 12 amino acids, i.e., the same sequence as the incorrect 14 amino acid sequence reported in the application minus the last two amino acids (TG). Similarly regarding the other error, the actual L19 antibody prepared prior to the Filing Date has and always has had a phenylalanine (F) in position 32 of its VL domain and not a tyrosine (Y) as reported in the application.

8. One of skill in the field of single chain antibodies as of May 11, 1998, could have used the procedures detailed in Examples 1 and 2 of the application filed on that date to arrive at, if not an antibody having the actual sequence of L19, then one having an equivalent specificity and affinity toward ED-B fibronectin. Nothing more than routine procedures are involved given the guidance of the specification. Examples 1 and 2 describe random affinity maturation procedures which statistically will achieve equivalent results in terms of functional characteristics (affinity, specificity, etc.) of the antibody isolated therefrom. Thus, when starting with the conventional human antibody library described in the first paragraph of Example 1 and following the procedures described in the remainder of Example 1, either the antibody E1 itself or a functional equivalent will be prepared. The following procedures of Example 2 describe how to mutate the sequences of E1 and a resultant higher affinity antibody is isolated (H10). Using the described specification details of this process, a skilled worker with no undue experimentation can readily mutate equivalent positions of the antibody obtained by the preceding Example 1 procedures to arrive at a functional equivalent of H10. In the next step of Example 2, positions equivalent to those mutated in H10 can be mutated in such functional equivalent of H10 to arrive at a resultant antibody having specificity and affinity equivalent to L19.

9. In the procedures of Examples 1 and 2, the mutated VH and VL regions are assembled conventionally by PCR (Example 1, lines 13-14 citing Clackson (1991)). These procedures were fully conventional as of May 11, 1998, as was the determination of suitable linkers. It was well known as of May 1998 that a wide variety of linkers could be employed successfully and nothing more than routine experimentation would be necessary for a skilled worker to find an appropriate linker in following Examples 1 and 2. (See, for example, Glockshuber, et al., Biochemistry 1990, 29, 1362-1367, reporting for a pair of certain VH and VL fragments linked using different linkers that "All the linked fragments show hapten affinities nearly identical to that of the whole antibody . . ." (Abstract).

10. In paragraph 2 of my prior Statement I referred to: "DNA encoding the antibody L19 as described in the above-identified application, e.g., as prepared in its Examples, particularly Example 2." In paragraph 4 thereof I stated: "The biological material which was

deposited at the American Type Culture Collection . . . is identical to the biological material specifically identified in the above-identified application as L19, as filed." As can be seen, neither statement was meant to imply that the sequence reported in the specification is that of an actual antibody which was in existence prior to the Filing Date. Nor could it reasonably be interpreted to mean this. This is because the sequence described in conjunction with Example 2 is erroneous and never existed in an actually prepared antibody. In context, these statements are meant to convey that the procedural steps used to prepare the actual antibody L19 are given in the Examples (irrespective of the inaccuracy in describing the actual sequences involved). As noted above, the deposited L19 antibody (PTA-9529) is identical in DNA sequence to the actual L19 which was in existence prior to the Filing Date.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

11 JANUARY 2011
Date

Dario Neri
Dario Neri

L19 – SEQUENCE

VH

EVQLL	ESGGG	LVQPG	GSLRL	SCAAS	GFTFS	SFSMS
WVRQA	PGKGL	EWVSS	ISGSS			
GTTYY	ADSVK	GRFTI	SRDNS	KNTLY	LQMNS	LRAED
TAVYY	CAKPF					
PYFDY	WGQGT	LVTVS	S			

LINKER

GDGSS	GGSGG	AS

VL

EIVLT	QSPGT	LSLSP	GERAT	LSCRA	SQSVS	SSFLA
WYQQK	PGQAP	RLLIY	YASSR	ATGIP	DRFSG	SGSGT
DFTLT	ISRLE	PEDFA	VYYCQ	QTGRI	PPTFG	QGTKV
EIK						